University of Zurich[UZH]

Master thesis

Effects of temperature, photoperiod and developmental stage on diapause in black scavenger flies (Diptera: Sepsidae)

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December 2015

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Contents

Abstract

Temperature and photoperiod are the major environmental cues used by insects to determine the best timing to overwinter. However, the relative importance of each of these variables and the life stage at which individuals are sensitive to diapause induction cues remains largely unresolved. Furthermore, it is not known how diapause-induction mechanisms differ between closely related species. Here, I addressed these questions using five species of black scavenger flies (Diptera: Sepsidae: *Sepsis*), which are increasingly used as model organisms in phylogeny, ecology and evolutionary biology. To disentangle the effects of temperature and photoperiod, I assigned females to four treatment combinations in which I varied temperature and photoperiod. Additionally, to study a putative life stage effect, flies were assigned to the different treatment combinations at four life stages (larval, pupal and two adult stages). I show that even closely related species use different environmental variables as diapause inducing cues and that these cues act independently of the life stage during which the varying environmental conditions are experienced. This observation thus highlights the adaptability of the diapause-induction mechanism. Although diapause-induction mechanisms were found to contain a strong phylogenetic signal, they also corresponded to the species' adapted thermal niches. Furthermore, I observed a strong adaptive signal in diapause-induction mechanism between two sister species. Additionally, in order to determine the diapause-induction dynamic in both sexes of *S. cynipsea*, I conducted a seasonal field sampling. Testis-size was used as a diapause surrogate in males. No change proportion of diapause incidence in females or testis size allometry was observed across the seasonal sampling.

Key words: diapause, diptera, species-specific induction, phylogenetic signal, thermal adaptation

Résumé

A l'approche de l'hiver, dans le but de définir de façon précise le moment opportun pour entrer en diapause, il est connu que les insectes se fient principalement à deux signaux environnementaux : la température et la photopériode. Cependant, l'importance relative de ces deux variables environnementales ainsi que durant quel stage développemental les insectes sont sensibles à de tels signaux demeure inexpliqué. En outre, la façon dont le mécanisme d'induction de la diapause varie entre espèces possédant un patrimoine génétique très semblable n'est pas connu. Dans cette étude, nous tentons de répondre à ces questions à l'aide de cinq espèces de Sepsidae (Diptera : Sepsidae : Sepsis). Cette famille est de plus en plus utilisée en tant qu'organisme modèle en phylogénèse, écologie et biologie évolutive. Afin d'étudier l'effet de la température et de la photopériode, nous avons assigné les femelles à quatre traitements dans lesquels nous avons fait varier la température et la photopériode. De plus, dans le but d'étudier un possible effet du stage développemental sur l'induction de la diapause, les mouches ont été transférées dans les différentes combinaisons de traitement à quatre stage développementaux distincts (larvaire, pupal et deux stages adulte). Ici, nous démontrons que pour induire la diapause, même des espèces génétiquement très proches utilisent les variables environnementales de manière différente et que ces variables ont un effet indépendamment du stage développemental durant lequel elles sont expérimentées. Tout ceci souligne l'importante évolutivité du mécanisme d'induction de la diapause. Bien que nous ayons observé que les mécanismes d'induction de la diapause affichaient un fort signal phylogénétique, il s'est avéré qu'ils correspondaient également à la niche thermale auxquelles ces espèces étaient adaptées. En outre, un fort signal adaptif a été observé chez deux espèces jumelles. Par ailleurs, dans le but de déterminer la dynamique d'induction de la diapause pour les deux sexes chez *S. cynipsea*, sur le terrain, nous avons effectué un échantillonnage saisonnier. Nous avons utilisé La taille des testicules comme estimateur du statut de la diapause. Aucun changement dans l'incidence de la diapause chez les femelles, ni dans la relation allométrique de la taille des testicules n'a pu être observé durant l'échantillonnage.

1. Introduction

1.1 The adaptive significance of diapause

Insects living in temperate regions have to cope with seasonal cycles, including harsh winter temperatures, suboptimal reproduction conditions and periods of food shortage. The evolutionary responses to these challenges encompass changes in behavior, physiology or metabolism and can be grouped as temporal and spatial avoidance strategies referred to as diapause (Danks, 1987). Diapause is a polyphyletic trait that permits insects to synchronize their life-cycle to the optimal environmental conditions and can be induced by either environmental or genetic factors (Tauber *et al.*, 1986). Diapause-induction results in a dramatic reduction in metabolic activity and allows insects to avoid adverse time and energy losses by entering a deep dormancy state. Danks (2002) defined diapause as "*A process helping individuals in many ways: conserving energy, protecting against adversity, synchronizing individuals with one another or with food resources, optimizing the timing of reproduction, monitoring environment for longer periods and preventing development at risky times of year*". Diapause is not a state that can be spontaneously switched on and off. Once induced, in order to avoid an untimely awakening, diapause will always go through successive species-specific phases until the termination phase (Kostál, 2006). Only when terminating conditions are present and other conditions have been fulfilled (e.g. the insect has experienced adverse-season-like conditions – akin to vernalization in plants), will the insect restore its full physiological activity. The existence of mandatory terminating conditions is a special attribute that distinguishes diapause from other states of dormancy, such as quiescence and stratification. The impressive number of features associated with diapause makes it one of the processes that has a major effect on insects life history and explains the maintenance of energetically costly regulatory and sensory diapause-triggering machinery (DeWitt *et al.*, 1998). In an unpredictable environment, the apparent cost of this machinery is outweighed by the advantage of a highly adaptive diapause-induction. This was confirmed by recent theoretical models showing that fluctuating seasonal conditions result in the maintenance of adaptive induction of diapause (Kivelä *et al.*, 2013).

1.2 Environmental inducing cues

A unique aspect of diapause is its anticipatory nature: given specific cues announcing the arrival of adverse environmental conditions, the insect will undergo major metabolic changes. The most regularly reported diapause environmental induction cues are temperature and photoperiod (Denlinger, 1972; Danks, 1987). Photoperiod (i.e. the number of hours of light per day) is a reliable cue as it follows a circannual cycle (Wilde, 1962), is used by a wide range of organisms to synchronize their development in a seasonally constrained environment (Hastings, 2001; Bradshaw & Holzapfel, 2007), and has often been reported as the major diapause-inducing environmental cue (Danks, 1994). Temperature exhibits important seasonal variations and is therefore a less informative seasonal-timing cue. It would, however, be misleading to minimize the effect of temperature on diapause-induction; in ectotherms, temperature has a major influence on the timing of diapause-induction, the development time and influences the number of generations produced per year (Huey & Kingsolver, 1989; Altermatt, 2010). Its influence is especially important in multivoltine species (species producing more than two generations a year) in which temperature is the source of a bet-hedging pathway to produce additional generations under favorable conditions. In the context of global warming, changes in voltinism have already been described (Altermatt, 2010) and models projecting an increase of mean surface temperature (>2°C) forecast dramatic changes in insect voltinism (Tobin *et al.*, 2008). An increase in the average number of generations per year could result in accelerating evolutionary changes and ameliorate the adaptability of insects to a changing environment (Altermatt, 2010). Although the influence of both temperature and photoperiod has been widely documented, the relative importance of both environmental cues in diapause induction remains unresolved (Tobin *et al.*, 2008). Gaining insights into this process, however, is crucial in order to acquire a better understanding of insect phenology and their potential to adapt in response to changing climate conditions (Chown *et al.*, 2010).

1.3 Reproductive diapause

Insects display a broad range of metamorphic stages entering into diapause: egg-, larval-, pupal- and adult-diapause have all been reported (Bell *et al.*, 1975; Bean, D. W., Beck, 1980). The life stage sensitive to diapause inducing cues can significantly vary across species and is always prior to the life stage entering into diapause. This may also involve maternal (or even grand maternal) effects (Mousseau, T. A., & Dingle, 1991). Insects overwintering at adult stage frequently undergo reproductive diapause, which is defined as "*the arrest of development of oogenesis, vitellogenesis, accessory gland activity, and mating behavior*" (Tatar & Yin, 2001). Particularly for females, the production of eggs is extremely energy demanding (Bateman, 1948). Thus, in order to survive harsh winter conditions, females need to save energy and invest instead in lipid production and energy storage (i.e. diapause pathway) rather than spend it for egg development (i.e. reproduction pathway, [Tauber *et al.*, 1986]). This has been extensively studied in species belonging to the Drosophila lineage (Schmidt *et al.*, 2005b; Schmidt, P. S., & Conde, 2006), and may also represent the dominating process occurring within the family of Sepsidae, where laboratory experiments have suggested absence of diapause at the egg, the larval and the pupal stage (Blanckenhorn, 1998).

1.4 Sepsid flies and research questions

The Sepsidae family is comprised of approximately 300 species, with the majority of them being coprophagous and playing an important biological role in the dung fauna (Ozerov, 2005; Ang & Meier, 2010). Sepsids are widespread throughout Europe and inhabit a wide range of climate conditions, making them highly suited to study diapause-induction mechanisms. Furthermore, the availability of a well-resolved phylogeny (Zhao, et al., 2013), together with detailed information on geographic distribution patterns (Pont & Meier 2002, Rohner et al. 2015), as well as thermal tolerance curves (Berger et al., *unpublished*) makes comparative analyses particularly powerful to study diapause induction mechanisms in a phylogenetic and an ecological context. Grasping this remarkable study framework, I carried out a meticulous selection of five species, all belonging to one genus occurring in Europe: the *Sepsis* genus. The species' selection allowed me to study the diapause-induction mechanism in (1) species associated with variable thermal niches and with different levels of relatedness. Giving this framework, in order to study (2) the relative importance of diapause-inducing environmental cues, flies were assigned to four treatments combinations in which I varied temperature and photoperiod. Furthermore, (3) to determine during which life stage Sepsid flies are sensitive to diapause-induction, flies were assigned to the latter treatments at four life stages (larval, pupal and two adult stages).

Additionally, in the scope of exploring the phenology of diapause near the putative end of the season, I dissected the reproductive organs of a large number of field sampled *S. cynipsea* of both sexes. In particular, I hypothesized that (1) the proportion of females in diapause should decrease across the season and (2) that males should display a differential reproduction investment toward the end of the season.

2. Material and methods

As outlined above, this study consisted of two complementary parts. The first part addressed the relative contribution of temperature and photoperiod on diapause induction in five Sepsid species. This experimental setup also examined the sensitivity to diapause induction at four discrete developmental stages. In the second part of the study, I conducted a seasonal field sampling of *S. cynipsea*. The purpose of the field sampling was to study the diapause-induction dynamic in both sexes across the end of *S. cynipsea*'s active season (June – October). In the following sections, I will first describe the details of the comparative experimental approach and then outline the details of the field study.

2.1 Choice of species

For the comparative experimental study, I used five species of the temperate *Sepsis* genus with known phylogenetic relationship, which are adapted to different thermal (ecological) niches (Fig. 1, [Pont & Meier, 2002; Ozerov, 2005]). The choice of species included: *Sepsis cynipsea* (Linnaeus, 1758)*, S. fulgens* (Meigen, 1826)*, S. neocynipsea* (Melander & Spuler, 1917)*, S. punctum* (Fabricius, 1794) and *S. thoracica* (Robineau-Desvoidy, 1830 [for a phylogenetic, see Fig. 2]). In Europe, *S. neocynipsea* and *S. cynipsea* are rather cold adapted, whereas *S. thoracica* appears to be warm adapted as it occurs at high density only in southern Europe. Similar applies to *S. punctum,* which is restricted to lowland environments in Switzerland. The avoidance of cold climates in *S. punctum* is also reflected by the strong genetic separation between northern and southern populations of the Alps (Puniamoorthy, 2013). *S. fulgens* appears to be a thermal generalist and occurs at high abundance across the entirety of Europe. Within Switzerland *S. fulgens* also shows no clear altitudinal distribution pattern.

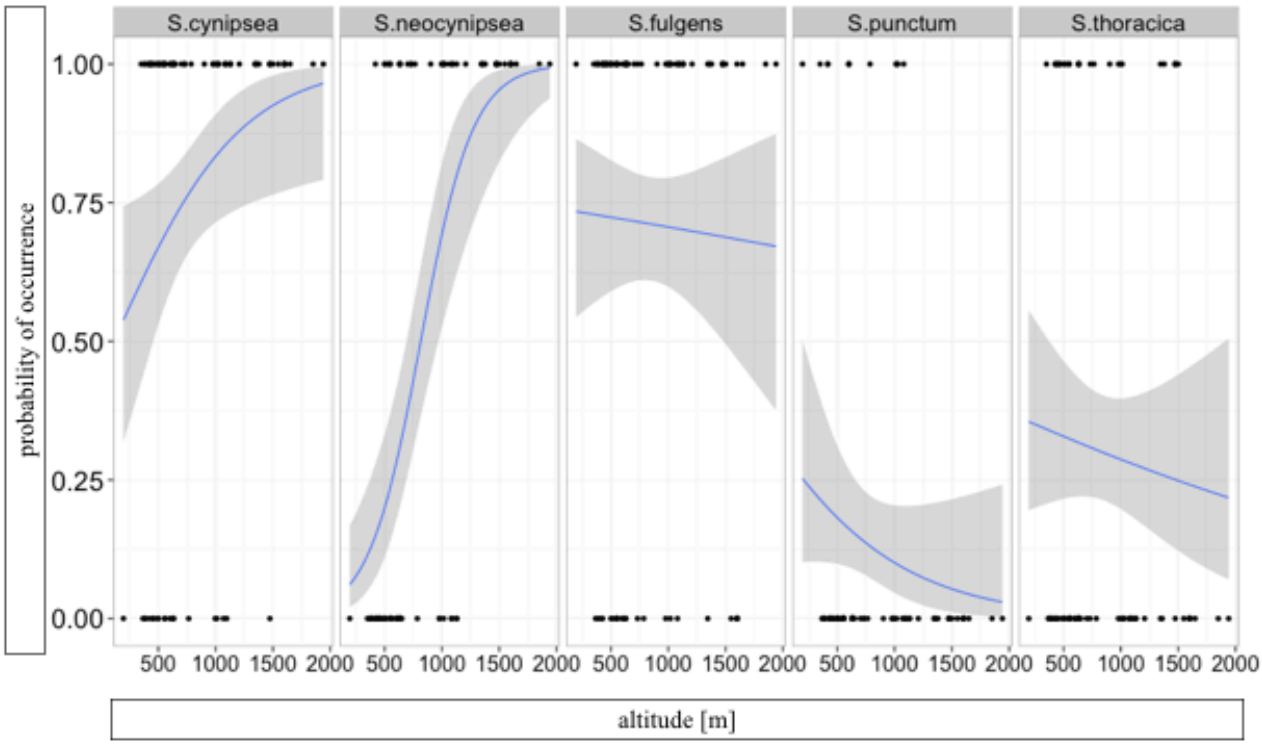


Figure 1: Probability of occurrence [%] within each sampling location of studied species in function of sampling altitude [m] (grey zone: 95% CI). High altitude sampling-localities displayed higher occurrences of *S. cynipsea* and *S. neocynipsea* as *S. punctum* and *S. thoracica* were associated with lowlands pastures. *S. fulgens* exhibit a ubiquitous distribution. Adapted from (Rohner *et al.*, 2015).

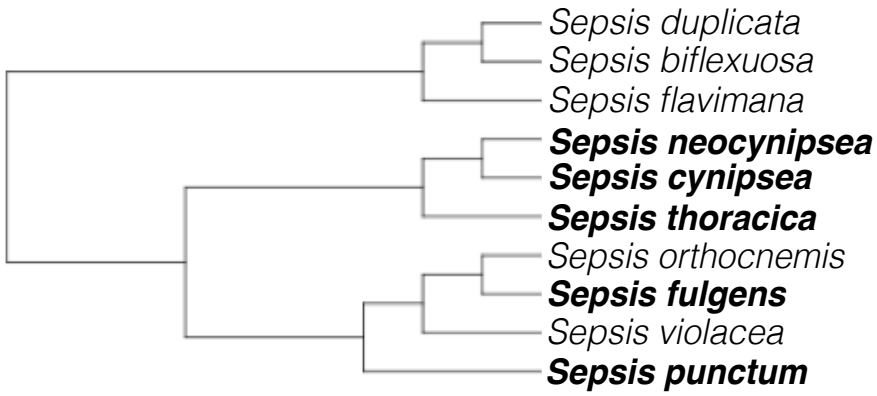


Figure 2: The most recent phylogeny of the *Sepsis* genus (Diptera: Sepsidae), proposed by (Zhao *et al.*, 2013). In bold, species of the *Sepsis* genus studied in this project. Notice the various degrees of relatedness among the studied species as well as the sister species: *S. cynipsea* and *S. neocynipsea*.

2.2 Rearing conditions

Prior to the experiments, isofemale lines of the five species of interest (Fig. 2) were cultured in a controlled climate chamber at 18°C and 12 h light:12 h dark. Isofemale lines originated from a single wild-caught female and were bred for several generations in the laboratory prior to be used for the experiments. The high degree of genetic relatedness within lines minimizes confounding effects of laboratory adaptations (Harshman & Hoffmann, 2000), but maintains genetic variation within populations. Each isofemale line was maintained in plastic containers (17 x 7.5 x 7.5 cm) with ad libitum cow-dung, sugar and water. For all species, cow-dung was provided as both a natural protein source, essential for the production of eggs (Pont & Meier, 2002), and as the ovipositing substrate in which the larvae can develop.

Before conducting the experiments, I performed outcrossing manipulations, by crossing Swiss populations in order to minimize the effect of latitudinal and altitudinal variation in diapause phenology (as reported in several species of flies: Blanckenhorn, 1998; Demont & Blanckenhorn, 2008; Schmidt, Matzkin, Ippolito, & Eanes, 2005). These outcrossing manipulations have the advantage of increasing inter-individuals genetic variation and limit possible maternal effects. For each of the five Sepsis species studied, two-six different populations were selected, among which, one to four lines (i.e. isofemale lines) were selected. For each selected lines, 20 individuals were randomly gathered into a single 24 x 12 x 12 cm plastic container, these selected flies were raised for two generations. Each line and population selection (Appendix: Table A1) was randomly computed according to the available material.

For the experiments, flies obtained after conducting the outcrossing manipulations were allowed to mate for 48 h (generation O). Dung-pots were then replaced and flies from generation O were allowed to oviposit for an additional 48 h. Dung-pots in which eggs had been oviposited were then transferred into a container (parent flies were thus removed). Four days after emergence, when F1 generation reached sexual maturity, F1 flies were allowed to oviposit in new fresh-dung during 48 h. Dung-pots in which eggs had been oviposited (F2) were stored in the controlled climate chamber (18°C, 12 h of light/12 h of dark). At the different life stages corresponding to their treatment (Fig. 3), 40 flies of the F2 generation were randomly selected and assigned to one of the four treatments (in an incubator).

At the emergence of F2 individuals (depending on the stage assignment: in the controlled climate chamber or in the incubator), 13 couples from each treatment combination were transferred into individual 8 x 2.5 x 2.5 cm vials containing *ad libitum* cow-dung, sugar and water. Couples were held together to increase female's fertilization chances. Indeed, mating activity could have an influence on female's egg production and thus, on the resulting diapause phenotype. Couples were first allowed to feed on fresh cow-dung for 96 h, giving the female enough time to accumulate energy to develop eggs. After these 96 h, as two of the studied Sepsids species (*S. cynipsea* & *S. neocynipsea*) exclusively oviposit in fresh media (Pont & Meier, 2002), the cow-dung pot was replaced. Couples were kept in the individual glass vials for nine additional days, this period allowing females to develop eggs. After 13 days (4 + 9), flies were killed by a three minutes passage in a -80°C freezer and individually stored at - 20°C in individual empty *Eppendorf* tubes, for later diapause assessment.

2.3 Experimental design

In order to explore differences in diapause induction mechanisms, I performed a full factorial experimental design in which I varied temperature and photoperiod, resulting in four temperature and photoperiod treatment combinations (Fig. 4). Flies of each species were assigned to four incubators (SANYO, MIR-154). The incubators were set to the following temperature and photoperiod treatments: (I) 18°C, 16 h light: 8 h dark (*warm/long*); (II) 18°C, 8 h light: 16 h dark (*warm/short*); (III) 12°C, 16 h light: 8 h dark (*cold/long*) and (IV) 12°C; 8 h light: 16 h dark (cold/*short*). Temperature in the incubators varied ±1°C and relative humidity was set constant at 60%.

Temperature treatments were chosen in accordance to the average records in Zürich (Switzerland) during the 1904-2004 period (Bey *et al.*, 2011). The average temperature in Zürich (alt. 408m) is 17.13±1.07°C in summer and 9.15±1°C in autumn. The warm temperature treatment (18°C) was set 1°C higher than the average temperature in summer to make sure that this condition will not induce diapause. The 12°C treatments was chosen as the best balance between a very long development time (at lower temperatures) and a high likelihood of diapause induction (at higher temperatures). Concerning the photoperiodic condition of the *short* (8 h light:16 h dark) treatment, it is never reached in Zürich (47°N 8.3°E); where the

longest and shortest days of the year are respectively 15h42min and 8h12min. Thus, this treatment should fall under the critical photoperiod (i.e. the photoperiod at which 50% of the individuals follow a diapause pathway and 50% are follow a development pathway).

The warm/long treatment was designed to provide benign control conditions in which flies follow the developmental pathway as the cold/short treatment should reproduce environmentally stressing conditions, in which flies follow the diapause pathway. The warm/short and cold/long treatments are nonsensical and are not an attempt to reproduce natural conditions. They will thus permit to disentangle the relative importance of temperature and photoperiod.

In order to examine the extent to which diapause-induction depends on the life stage during which diapause-inducing environmental cues are experienced, flies were assigned to the temperature and photoperiod treatment combinations at the following life stages (Fig. 3): (1) immediately after oviposition (*larval*); (2) at the beginning of pupal stage (*pupal*); (3) at adult emergence (*emergence*) and (4) one week after adult emergence (*one week old adult*). Our experimental design thus resulted in 16 treatment combinations (Fig. 4) per species (2 temperatures × 2 photoperiods + 4 × life stages).

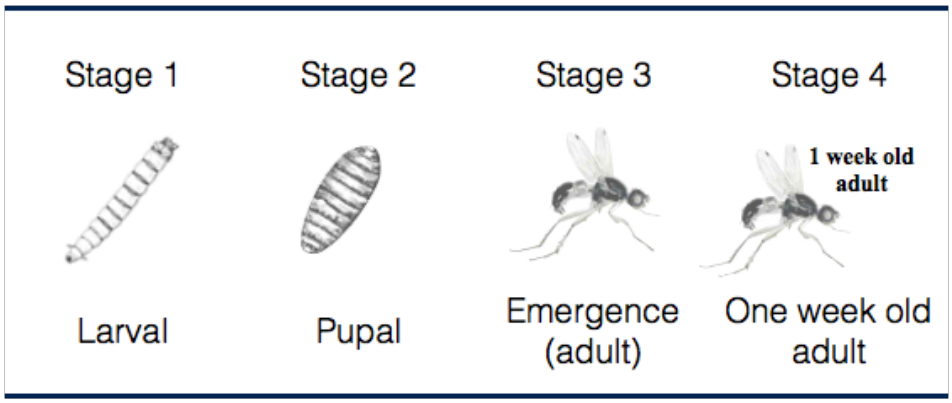


Figure 3: Flies were assigned to the temperature and photoperiodic treatments at four discrete life stages: (1) immediately after oviposition (larval); (2) at the beginning of pupal stage (pupal); (3) at adult emergence (emergence) and (4) one week after adult emergence (one-week-old adult).

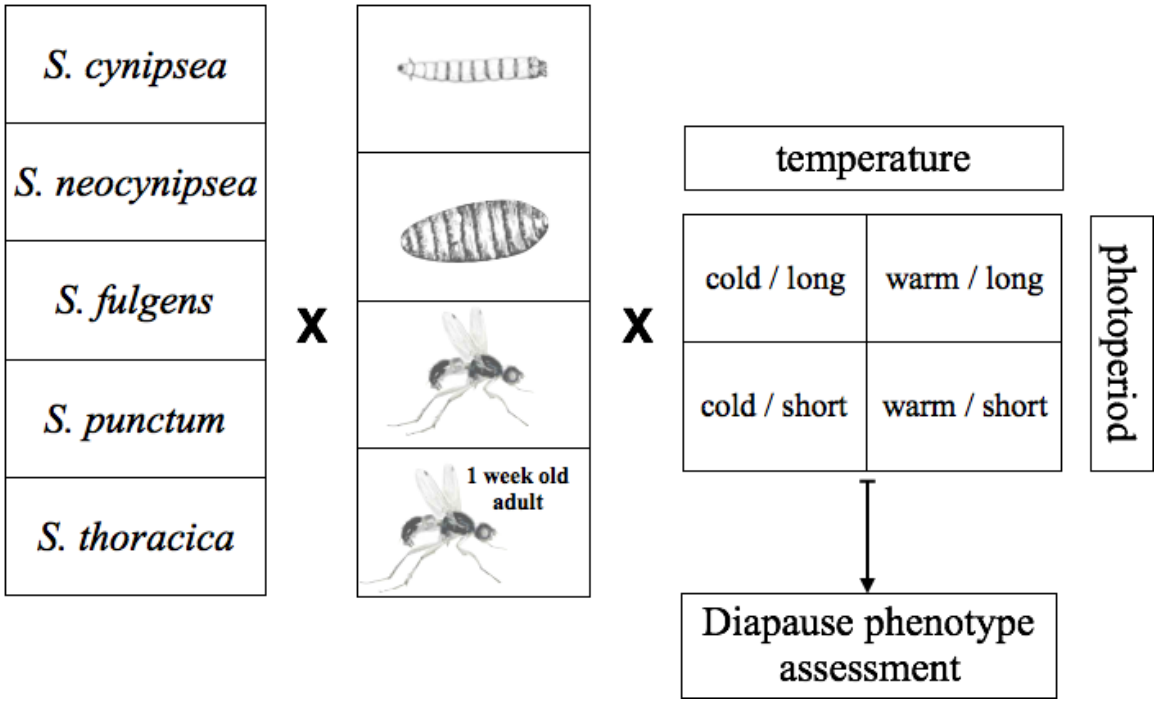


Figure 4: Experimental design: five species of the *Sepsis* genus were assigned at four discrete life stages (larval, pupal and two adult stages) to four temperature (cold: 12°C; warm 18°C) and photoperiod (short: 8 h light/16 h dark; long: 16 h light/8 h dark) treatment combinations. After 13 days, females were dissected to assess their diapause phenotype.

2.4 Female diapause assessment

To assess their diapause phenotype, females were dissected under a microscope (Leica MS 5, magnification: x32). Reproductive diapause in Sepsid flies can be determined by the developmental stage of the eggs in the ovaries (Appendix, Fig. A1). To reduce potential observer-bias, scoring was performed without knowledge of the experimental treatment (blind assessment). This process was destructive and samples were discarded after the assessment. Additionally, as an estimator of body size, forelegs of all samples were placed on a microscope slide in *Euparal*. These microscope slides were photographed using the Leica Application Suite (LASV4) and the foretibia length of one leg was measured using the morphometric software tpsDIG2.

2.5 Seasonal field sampling

Seasonal sampling of *S. cynipsea* was conducted over a period of ten weeks in a field in *Ziegelhütte* (Zürich, Switzerland [47°23' N 8°34' E]), every three weeks from June to August and every week between August and October 2015 (i.e. from beginning of Summer to mid-Autumn). Photoperiod during the seasonal sampling ranged from 16 h light:8 h dark in June to 11.5 h light: 12.5 h dark in October.

The seasonal sampling was carried out using two captures methods: (I) catching at least 40 couples of *S. cynipsea* near fresh-dung pad using an exhaustor (couple-caught) and (II) performing five segments of 50 m across the field using an insect-net (net-caught). The couple-caught method was motivated by the absence of unambiguous external characteristics in females, rendering their taxonomic identification laborious (Rohner *et al.*, 2015).
Identification issues concerning females were thus resolved by capturing flies while they were mating, ensuring the capture of a female of the correct species. I decided to focus on *S. cynipsea* because of female's shaking behavior, which is one of the major feature of the species' mating habit, making them easily to recognize (Parker, 1972; Teuschl & Blanckenhorn, 2007). Additionally, low genetic differentiation between Swiss *S. cynipsea* populations has been reported (Kraushaar *et al.*, 2002), reducing thus the risks of population's biased observations. Due to the aforementioned identification issues, only *S. cynipsea* males were captured with the net-caught method. After sampling, flies were then killed by a three minutes passage in a - 80°C freezer in the laboratory. Couple-caught samples were immediately stored in *Eppendorf* tubes while net-caught *S. cynipsea* males were first identified and then stored.

2.5.1 Field-samples diapause assessment

Couple-caught females were dissected and foretibia length was measured following the same procedure as for the lab-sample. Whereas testis size allometry was used as a putative diapause estimate for males (from both capture methods). The meticulous testis dissection procedure (Fig. 5) was performed under the microscope. Testis area was measured using the software tpsDIG2; four landmarks were taken on each extremity of the testis (the longest and the widest part). The area of each testis was then estimated (mm^2) by fitting an ellipse.

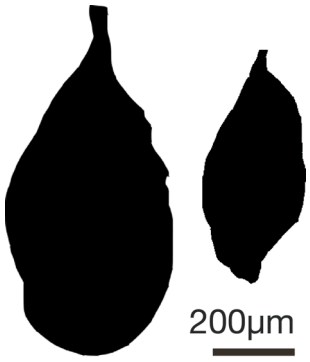


Figure 5: Illustration of variation in testis size between two *S. cynipsea* males.

2.6 Statistical analyses

Experimental data were analyzed in two steps using multiple logistic regression (Janzen & Stern, 1998). First, in order to disentangle the relative importance of temperature and photoperiod on diapause-induction, the analysis was conducted without including the life stage assignment (Table 2). Within each species, differences in proportion of flies entering into diapause were tested with a generalized linear model (GLM, based on family binomial) using a logit link function with treatment (temperature, photoperiod and the resulting interaction) as fixed factor. Confidence intervals (95% CI) were obtained using a binomial proportion confidence interval (method: Wilson). The reference levels used by the model for the temperature and photoperiod treatments were respectively *warm* (18 °C) and *long* (16 h light: 8 h dark).

Second, seeking a putative life stage effect within species, I conducted separate analyses for each species (Table 3), the GLM and the CI were the same type as mentioned above. The four life stages studied are a succession in the insect's life and were coded as covariates (not as factors). I tested for a life stage effect beginning with temperature, photoperiod and life stage assignment as fixed factors. Non-significant variables were eliminated from the final analyses using a stepwise backward elimination procedure (Hosmer & Lemeshow, 2000). This elimination procedure included all three-way and most two-way interaction terms. The final

model retained photoperiod, life stage assignment and the resulting interaction as explanatory variables. Field data were analyzed with an analogous model with female diapause incidence and testis size as response variables. Because of its major biological relevance (Peters, 1985), I tested the effect of the body size estimate (foretibia length) on diapause incidence. Foretibia length was never significantly associated with diapause incidence and was therefore removed from the models.

Statistical analyses with binary outcomes are difficult to deal with (Bewick *et al.*, 2005). Logisitic regression is particularly sensitive to lack of variation, which mainly arises from the fact that the response variable is restricted between 0 and 1. Because some individuals raised at the warm/long treatment or cold/short photoperiod treatment showed lack of variation in diapause incidence, I artificially added conservative variance in the subset with no variation (e.g. diapause incidence is equal to 0 in the *warm* and *long* treatment in *S.* thoracica, Fig. 7). In subset of data containing only "0", one "1" was added and vice-versa. In total, 31 data-points were added (Appendix: Fig. A2).

All statistical analyses were performed using RStudio[1] version 0.98.1062 and SPSS[2] version 22.0. All significant thresholds were two-tailed and set to $\alpha=0.05$.

[1] R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

[2] IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

3. Results

3.1 Effects of temperature and photoperiod across species

Temperature and photoperiod strongly affected diapause induction in all species (Fig. 6, Appendix: Table A1). Most females of all studied species entered into diapause in the *cold* and *short* treatment, although diapause incidence varied slightly among species. Also, all species developed eggs under *warm* and *long* conditions. However, diapause-induction between species differed considerably in response to the nonsensical treatments (*warm/short* and *cold/long*). These results demonstrate striking variation in diapause-induction mechanisms among closely related species. The cold-adapted sister species *S. cynipsea* and *S. neocynipsea* developed eggs in the *warm* and *long* treatment only, and went into diapause in the *cold* and *short* treatment as well as in the nonsensical treatments (Table 2, Fig. 6). On the other hand diapause in the warm-adapted species *S. punctum* and *S. thoracica* was induced by temperature alone (Table 2, Fig. 6). The temperature × photoperiod interaction in *S. punctum* was only marginally significant (Table 2, Fig. 6). The thermal generalist *S. fulgens* showed no signs of reproductive diapause except under the cold/short treatment. This is supported by a significant temperature × photoperiod interaction term (Table 2, Fig. 6).

Table 2: Multiple logistic regression of diapause incidence within studied Sepsid species in relation to temperature and photoperiod and the resulting interaction. Reference levels for temperature and photoperiod were respectively: *warm* (18°C) and *short* (8 h light:16 h dark). Significant *p*-values are in bold. N: total sample size per species.

	df	*S. cynipsea*		*S. neocynipsea*		*S. fulgens*		*S. punctum*		*S. thoracica*	
source	1	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
temperature	1	4.7	**<0.001**	1.53	0.216	21.55	**<0.001**	21.93	**<0.001**	16.3	**<0.001**
photoperiod	1	33.45	**<0.001**	26.9	**<0.001**	0.011	0.916	2.7	0.1	0.005	0.946
temp × photo	1	0.86	0.354	4.19	**<0.001**	5.08	**<0.001**	3.2	0.074	0.28	0.6
N		186		196		181		166		157	

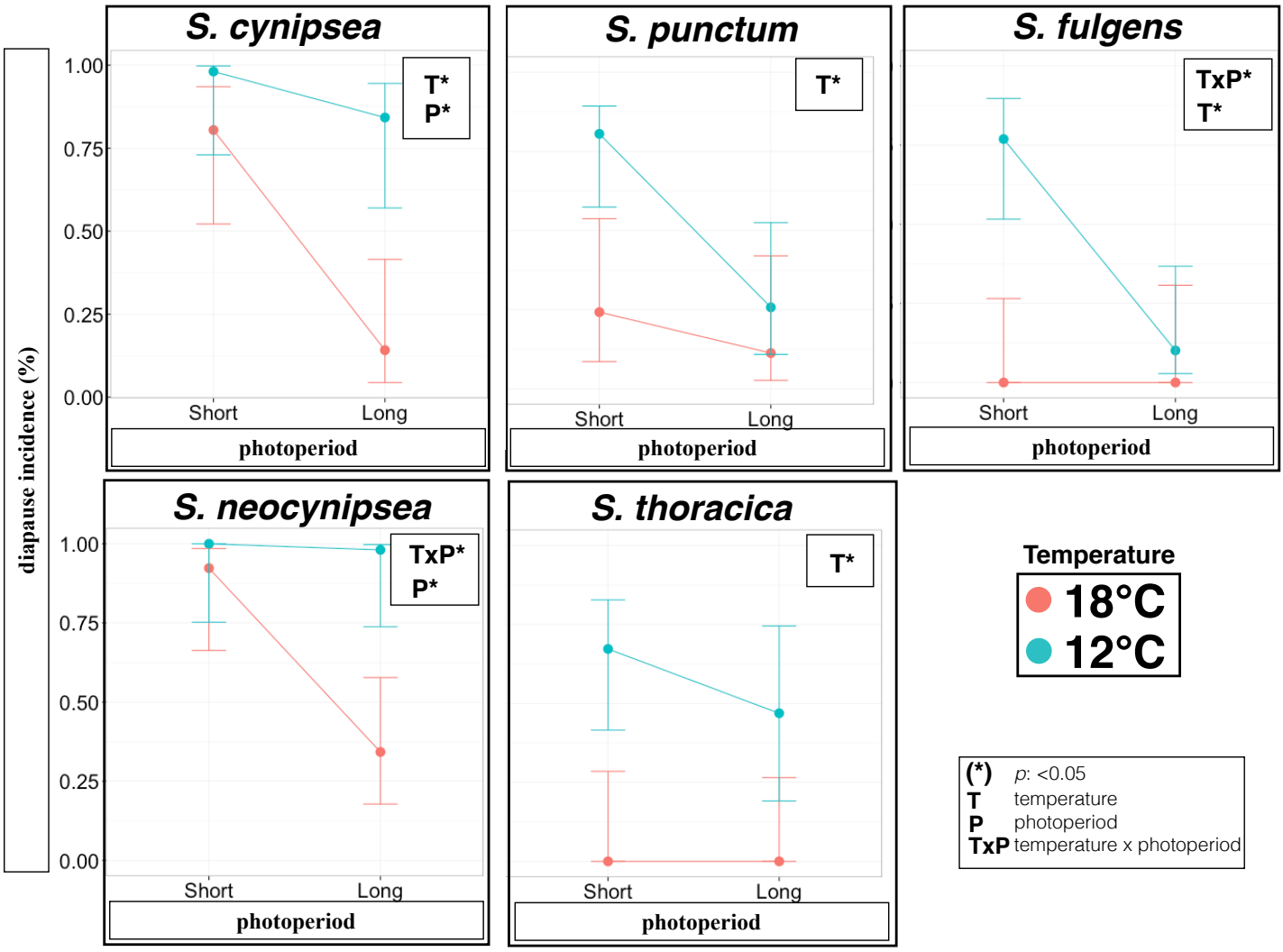


Figure 6: Diapause incidence (± 95% CI) within studied Sepsid species in relation to photoperiod and temperature. Variables associated with a significant change in diapause incidence are showed in the top-left boxes.

3.2 Life stage effects within species

I aimed to identify critical life stages sensitive to diapause-induction within each studied species. Multiple logistic regression did not detected any life stage effect across temperature and photoperiod treatments due to the lack of variation in some treatment combinations. For example, this was the case for the warm treatments for *S. thoracica*, *S. fulgens* and *S. punctum* and for the cold treatments in *S. cynipsea* and *S. neocynipsea* (Fig. 7). However, when only taking into account subset of temperature treatments displaying variation (cold treatments in *S. thoracica*, *S. fulgens* and *S. punctum*; warm treatments in *S. cynipsea* and *S. neocynipsea*), it was possible to detect a life stage effect (Table 3). The photoperiod × temperature interaction was removed from the models because it was only significantly associated with diapause incidence in *S. punctum*. The significance of this interaction is probably due to the missing data in the cold/short treatment (Fig. 7). The final within species model retained diapause incidence in relation to photoperiod and life stage assignment *within* temperature treatment (Table 3).

Within the cold treatments, diapause in *S. thoracica* was marginally induced by photoperiod and there was strong negative stage relationship (Table 3, Fig. 7). Always in the cold treatments, *S. punctum* was significantly affected by the single photoperiod effect (Table 3, Fig. 7) but no life stage effect could be detected. Within the warm treatments, there was a significant effect of photoperiod for both sister species *S. cynipsea* and *S. neocynipsea* (Table 3, Fig. 7). For *S. neocynipsea*, in the warm/long treatment, the life stage assignment exposed a peculiar positive quadratic relationship, resulting in a life stage effect detection (Table 3, Fig. 7). Both sister species were sensitive to photoperiod and temperature or an combination of both cues (Table 3). Always in the warm treatments, *S. fulgens* reacted to photoperiod and a marginal overall stage effect could be detected (Table 3, Fig. 7).

Table 3: Multiple logistic regression of diapause incidence within studied Sepsid species and temperature treatment in relation to photoperiod, stage assignment and the resulting interaction. Reference levels for temperature were respectively: first stage of assignment (larval) and *short* photoperiod treatment (8 h light:16 h dark). Significant *p*-values are in bold, N: total sample size per species and temperature. Complementary information for the interpretation of this table, see Appendix II (p.44).

			S cynipsea		*S neocynipsea*		*S fulgens*		*S. punctum*		*S. thoracica*	
temperature	source	d.f	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
cold	photo(1)	1	2.183	0.139	0.011	0.916	30.247	**<0.001**	13.691	**<0.001**	3.699	0.054
	stage	3	2.63	0.452	0	1	7.775	0.051	5.84	0.12	14.933	**0.002**
	stage(1)	1	1.941	0.164	0	0.998	1.947	0.163	0.17	0.68	11.202	**0.001**
	stage(2)	1	0.223	0.637	0	1	2.278	0.131	2.317	0.128	11.323	**0.001**
	stage(3)	1	0.031	0.86	0	1	0.123	0.726	1.857	0.173	5.504	**0.019**
N			98		105		101		87		74	
hot	photo(1)	1	30.847	**<0.001**	25.811	**<0.001**	0.061	0.805	1.709	0.191	0.018	0.892
	stage	3	3.38	0.337	9.558	**0.023**	0.3	0.96	2.286	0.515	0.005	1
	stage(1)	1	0.821	0.365	0.021	0.884	0.006	0.936	0.145	0.703	0.003	0.954
	stage(2)	1	2.7	0.1	5.751	**0.016**	0.002	0.963	0.205	0.651	0	0.993
	stage(3)	1	0.016	0.9	5.751	**0.016**	0.238	0.626	1.702	0.192	0.003	0.958
N			93		100		88		83		90	

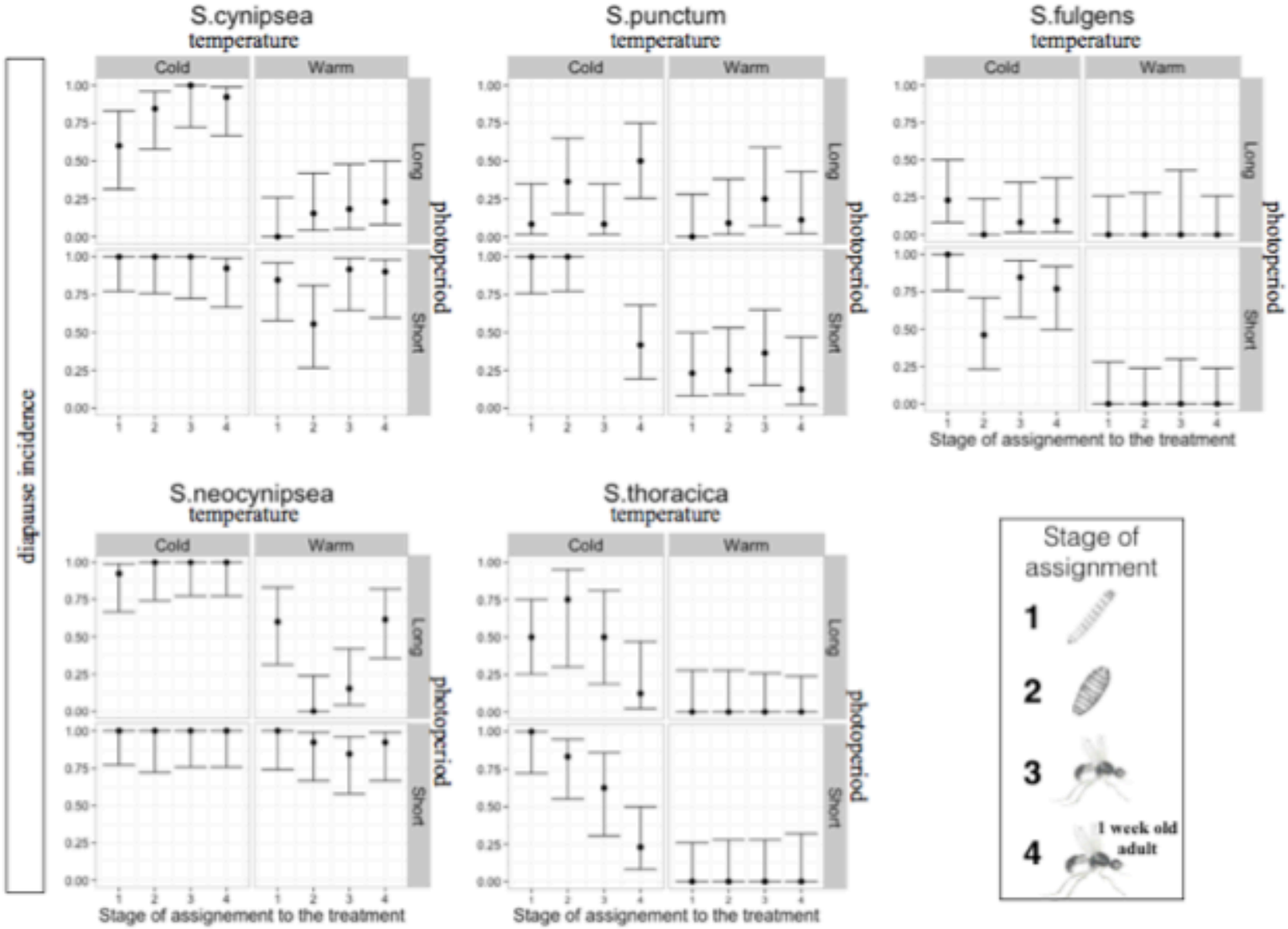


Figure 7: Diapause incidence (± 95% CI) within studied Sepsid species in function of photoperiod, temperature and life stage assignment. Key: warm (18°C) and cold (12°C); long (16 h light:8 h dark) and short (8 h light:16 h dark); for details concerning stage assignment, see Fig. 3.

3.3 Seasonal changes in diapause incidence in *S.cynipsea*

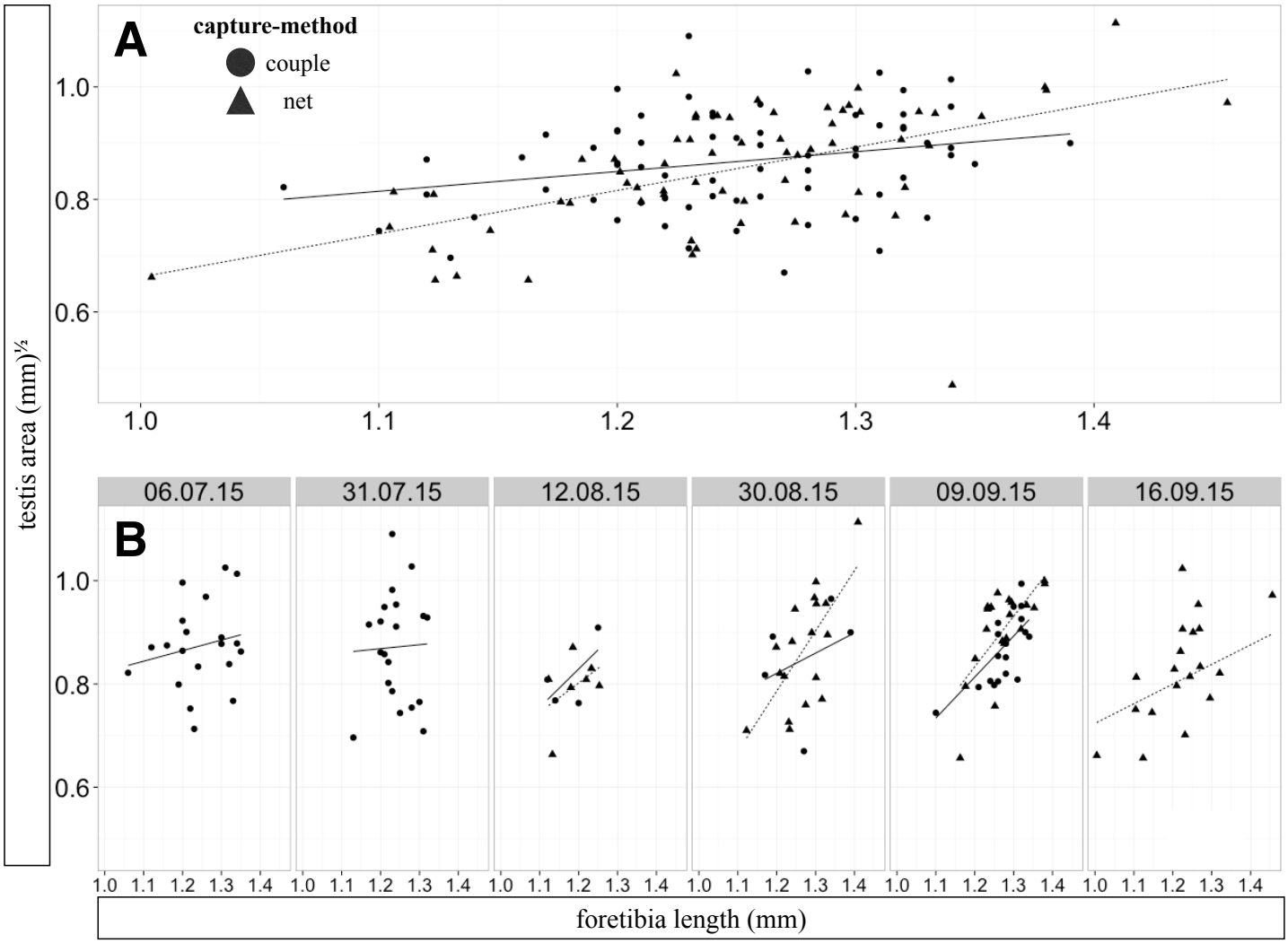
Over six different sampling dates, 69 *S. cynipsea* couples and 65 non-mating *S. cynipsea* males were caught and dissected in order to perform the diapause phenotype assessment. Not surprisingly, I observed an overall and within sampling date positive testis size allometry in males (Table 4, Fig. 8a, Fig. 8b). Overall sampling dates and within sampling-date, foretibia length by capture method interaction as well as the capture method effect were both marginally significant (Table 4, Fig. 8a). Contrarily to our expectation, no difference in testis size allometry was observed between the six sampling dates (Table 4, Fig. 8b). I observed a similar trend in females; diapause assessment of females caught around dung-pads revealed no systematic increase in the diapause incidence over the sampling season (logistic regression, Estimate: -0.047, p=0.96; Fig. 9).

Note that the field sampling with the net capture method was conducted from the 12 August and that no S. cynipsea couples could be found from the 09 September (Fig. 8b).

Table 4: Linear regression of testis size in relation to foretibia length and capture-method overall and within each sampling date. Reference levels for capture method was the couple-caught method. Significant p-values are in bold.

	overall the season				within sampling-date			
source	d.f	Estimate	t-value	P	d.f	Estimate	t-value	P
Intercept	1,129	0.43	2.09	**0.038**	1,124	0.45	2.19	**0.03**
Date (1st sampling date – 06.07.15)	5,129	–	–	–	5,124	-0.0049	-0.89	0.37
Capture method (net-caught)	1,129	-0.54	-1.97	0.051	1,124	-0.52	-1.9	0.059
Foretibia l. × Capture-method (net-caught)	2,129	0.42	1.92	0.057	2,124	0.42	1.9	0.053
Foretibia length	1,129	0.35	2.14	**0.033**	1,124	0.34	2.09	**0.038**

Figure 8: Linear regression of testis size ($mm^{1/2}$) as a function of foretibia length (mm) using two capture methods, (A) overall the sampling-season and (B) within sampling-date revealed a positive testis size allometry for both methods. No difference in testis size allometry was observed between sampling dates (B). Solid and dashed lines are resp. the regression line for the couple- and net-caught method.



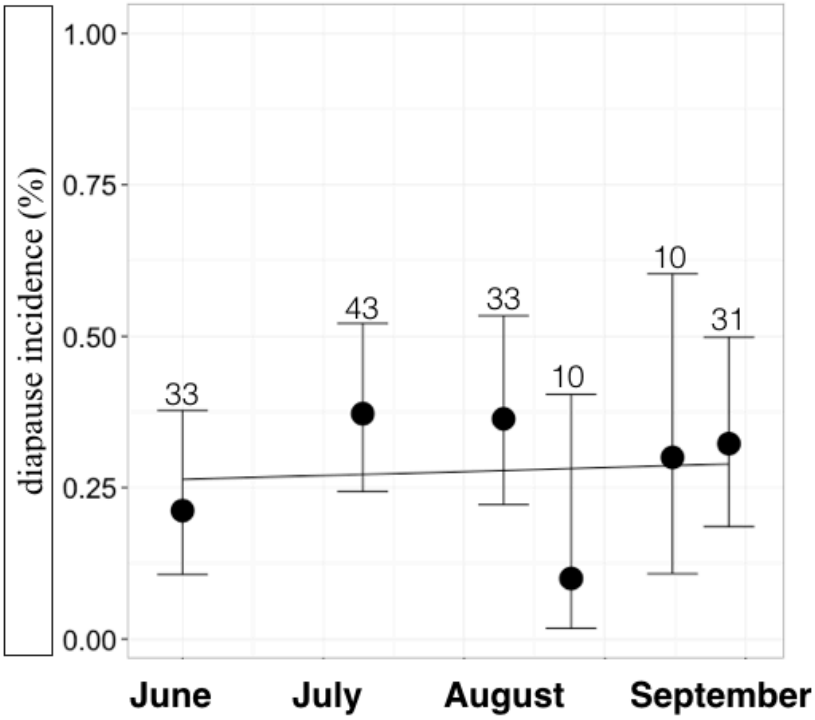


Figure 9: Logistic regression (± 95% CI) reports no variation in diapause incidence in females across the seasonal field sampling (Estimate = -0.002563, z-value= -0,505 p=0.613). Number above bar indicates sample size. Sampling dates, from left to right: 04.06.15, 06.07.15, 31.07.15, 12.08.15, 30.08.15 & 09.09.15.

4. Discussion

I studied the combined effects of temperature and photoperiod on reproductive diapause induction in females of five species of *Sepsis*. I further inquired the diapause-induction mechanism at four different life stages. My results show impressive variation in diapause induction mechanism within the genus, and suggest adaptive niche-specific responses. In addition, my data indicates a strong phylogenic signal in diapause-induction mechanisms as revealed by comparative analysis of sister species *S. cynipsea* and *S. neocynipsea*. Concerning our field-sampling, no systematic changes in diapause-induction towards the putative end of the reproductive season could be observed. Indeed, the proportion of flies going into diapause was rather constant in females (Fig. 9).

4.1 Influence of thermal niche on diapause-induction mechanism

Diapause-induction thresholds often vary considerably among species and even populations, revealing an adaption to local climate conditions (Tauber *et al.*, 1986). Within Switzerland, species of the *Sepsis* genus show heterogeneous distribution patterns, which are significantly influenced by local climate conditions (Rohner et al., 2015, see also Fig. 1). *S. punctum* and *S. thoracica* are mainly associated with lowlands environments suggesting a preference for warm temperatures. As sister species *S. neocynipsea* and *S .cynipsea* are present at high densities in highlands pastures, and are rarely found in Southern Europe, implying an avoidance of hot temperatures. *S. fulgens* seems to be a thermal generalist, exhibiting a homogenous elevational distribution and occurring throughout from Northern to Southern Europe (Pont & Meier, 2002; Ozerov, 2005). Interestingly, these differences in thermal niches mirror our findings on interspecies differences in reproductive diapause induction mechanisms (Table 2, Fig. 10). I found a highly significant effect of photoperiod (and also temperature) on diapause induction in *S. cynipsea* and *S. neocynipsea*. At high altitudes and latitudes, flies will always face adverse cold winter conditions. Under such circumstances it may be advantageous to rely on predictable long-term environmental cues like photoperiod rather than only short-term temperature fluctuations (Stephens, 1968). Relying on temperature alone would also result in a great fitness cost if diapause is induced every time temperature drops. Nevertheless, it is vital for the fly to avoid non-developmental friendly temperatures, making thus the usage of a combination of photoperiodic and temperature cues highly adaptive (Roff, 1983; Gomi, 1997). Warm-adapted species (*S. punctum* and *S. thoracica*) mainly responded to temperature cues, as reported for a range of other dipteran species (Denlinger, 1979; Roff, 1983; Scharf *et al.*, 2010). Contrary to high-altitude environments, lowlands regions show smaller temperature fluctuations, reducing the risk of unforeseeable lethal cold temperatures. Not relying on photoperiod alone allows a finer tuning of diapause-induction (Hairston & Kearns, 1995) and permits the extension of the reproductive season depending on temperature conditions (Altermatt, 2010). Regarding thermal generalists, I expected the optimal induction of diapause to be triggered by all environmental cues (Tauber *et al.*, 1986). This is consistent with our observations: *S. fulgens* was reproductively active under all treatment combinations, except under the cold temperature and short photoperiod regime.

4.2 Life stage effect

Several diapausing life stages have been reported in Dipteran species including the larval pupal (Saunders, 1971; Blanckenhorn, 1998) or ovarian life stages (Williams & Sokolowski, 1993). This suggests a great variation in diapause sensitivity with respect to different life stages. In the cold temperature treatments, I found a cumulative effect of the larval, pupal and adult stage on diapause induction in *S. punctum*, and *S. thoracica* (Fig. 7): i.e. the longer the individuals experienced cold-treatment conditions, the higher incidence diapause. Sampling information on seasonal timing cues across life-stages may be energetically costly, but allows organisms to predict changing environmental conditions more precisely than restricting the sampling period to a discrete developmental stage (DeWitt *et al.*, 1998). However, our experimental approach cannot disentangle the relative sensitivity of individual developmental stages. A way of solving this issue would require a punctuated exposure of discrete stages to the diapause inducing cues. Nevertheless, in *S. punctum*, this cumulative pattern was not consistent across all treatments. In cold and long conditions, this species displayed a positive life stage effect, which is difficult to interpret and may be elicited by the biological nonsensical conditions of this treatment. In *S. thoracica*, individuals assigned to cold temperature treatments after emergence also displayed increased proportions of diapausing females in comparison to their warm treatment counterparts (Fig. 7). Oosorption could be responsible for this phenomenon, suggesting that this species can still modify its reproductive status after emergence according to the environmental conditions (Bell & Bohm, 1975). Interestingly, life stage assignment did not affect diapause induction in *S. cynipsea*, *S. neocynipsea* and *S. fulgens*. This suggests that these species are sensitive to diapause-inducing environmental cues throughout their development and possess a lower threshold of diapause-induction than species associated with warmer thermal-niches. This interpretation of lower diapause induction thresholds is consistent with the finding that at least *S. cynipsea* and *S neocynipsea* showed higher diapause incidence values in most of the treatments and further underpins the fact that species being associated with environments with harsher conditions (e.g. high-altitude environments), dispose of a highly adaptive diapause-induction mechanism (Schmidt, P. S., & Conde, 2006).

4.3 Phylogenetic variation in diapause-induction mechanisms

There was strong intra-genus variation in the diapause-induction mechanism. In temperate environments, diapause undergoes strong selection and is one of the processes that has the most influence on insect's life-history (Tauber *et al.*, 1986). Interestingly, I nevertheless observed a strong phylogenetic signal as reflected by the high similarity in diapause-induction mechanisms between the sister species *S. cynipsea* and *S. neocynipsea*. A common diapause-induction mechanism not only indicates a shared genetic basis (as might be expected by the small pairwise distances at the CO1 barcoding gene < 1.34% reported in [Puniamoorthy, Ismail, Tan, & Meier, 2009]), but further suggests that the ecological and thermal niches (and co-adapted physiology) are quite conserved for certain periods of time. Future studies on thermal niche conservatism and associated diapause-induction mechanisms in an extended set of Sepsid species associated to a range of different thermal niches may help produce a better understanding of the adaptive potential to recent man-made climate change (Chown *et al.*, 2010).

4.4 Seasonal changes in diapause in *S. cynipsea* males and females

4.4.1 Males

Following the Y-model (Garland, 2014), I assumed a bet-hedging strategy in males, and expected a decreasing testis size allometry across the season. A bet-hedging strategy exists in individuals having a "*lower variance in fitness at the cost of a lower arithmetic mean*" (Hopper, 1999) and appears in environments with unpredictable conditions. This strategy is favorable when being highly adapted to only one type of environment result in a drastic fitness cost if the conditions change. Our data suggests that *S. cynipsea* males do not change their relative reproductive investment (here: testis size) across the season. However, it is still possible for a bet-hedging strategy to exist while remaining difficult to observe (Hopper, 1999). Producing sperm has long been considered to be less costly than producing eggs (Bateman, 1948), but this idea has been criticized numerous times (Tang-Martinez & Ryder, 2005; Snyder & Gowaty, 2007). Indeed, sperm production can be a limiting factor in many species including Sepsid flies (Teuschl *et al.*, 2010), leading to a trade-off: either invest in sperm or in survival (e.g. a lipid stock). The absence of any testis size allometry could also arise from the fact that when measuring testis size, it is actually the net effect of male' sperm production and sperm expense which is measured. Indeed, when copulating, males give sperm away, resulting in testis' size reduction and males produce sperm when foraging (Teuschl & Blanckenhorn, 2007). This lack of a clear diapause phenotype in males renders the study of diapause laborious and may hide putative differential in reproduction investment.

Alternatively, records of females entering diapause at the adult stage and males dying off have been reported in several orders (Tauber *et al.*, 1986). My inability to detect any differential testis size allometry across the season could arise from males that follow an all-in strategy: striving to increase their reproductive success and eventually die before winter. However, this is unlikely – as Sepsids from both sexes hatching after having been assigned to cold temperatures treatments from the egg stage have been reported (Blanckenhorn, *unpublished*). Additionally, surviving the winter can be considered advantageous for males fitnesswise, indeed;, it would be me much more risky to invest in a female before the winter (rather than after winter) due to the lower chance of female survival over-winter (Taylor, 1980).

4.4.2 Females

Contrary to our expectation, no difference in the proportion of females in diapause was observed across the season. Studies reported that the near dung-pad environment is primarily visited by reproductively active females (Teuschl, Reim, Meister, Egger, & Blanckenhorn, 2010; Teuschl, *unpublished*), making the sampling of females on the dung-pad environment unrepresentative of the females' whole population reproductive status. A way of circumventing this issue would be to also sample free-flying females, which is prohibitively tedious due to the lack of unambiguous females' structural traits, impeding a reliable species identification (Rohner *et al.*, 2015). This identification issue could be resolved by performing PCA on wing landmarks, which reliably allows one to distinguish females of different species (Baylac *et al.*, 2003).

These results leave us with a conundrum. The absence of any observed change in testis size allometry or diapause incidence in females across the season in the field suggest a patchy knowledge of Sepsid's life history. Diapausing individuals may withdraw to some other ecosystem (e.g. forest), leaving in the field only reproductively active individuals. One first step to gain better insight into *Sepsis* phenology in both sexes could be resolved by using outdoor field-cages, similarly to (Hoffmann, Scott, Partridge, & Hallas, 2003). This would resolve females' identification issue as well as permit a representative sampling of populations' reproductive status.

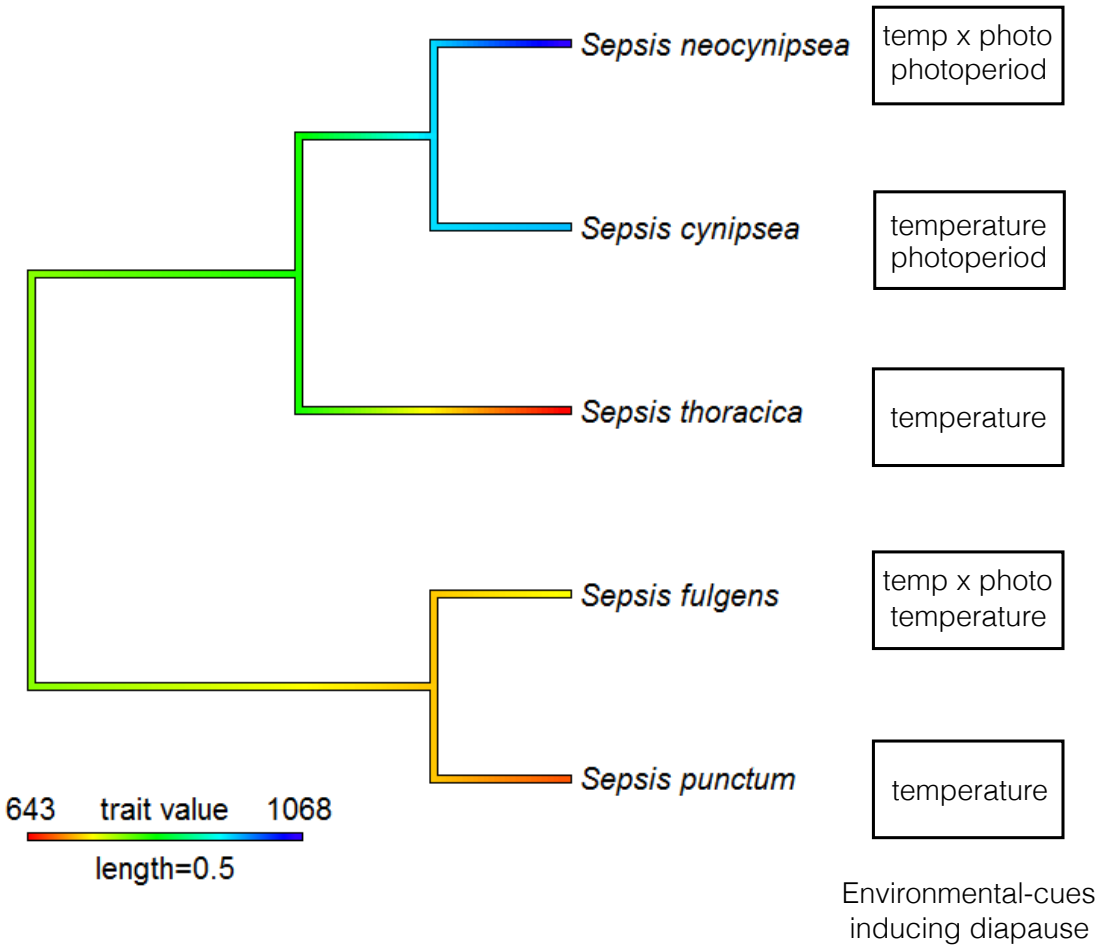


Figure 10: Ancestral state reconstruction using maximum likelihood of the median elevation of occurrence (cf. Fig. 1). The elevational distribution corresponds to the diapause-induction mechanisms. Cold-adapted sister species; *S. cynipsea* and *S. neocynipsea* are associated with high-altitude environments and induce diapause with temperature and photoperiodic cues; as warm-adapted species *S. thoracica* and *S. punctum* induce diapause with temperature cues. *S. fulgens* display a broad altitudinal distribution and induces diapause with temperature and photoperiodic cues. Adapted from (Rohner *et al.*, 2015).

4.5 Molecular outlook

The deterministic dimension of the genetic mechanisms underlying diapause induction has been pointed out by recent studies. The gene *couch potato*, which is functionally linked to a cline in diapause incidence has been reported (Schmidt *et al.*, 2008) and the gene *timeless* seems to be responsible for the circadian clock regulation in European species of Drosophilidae (Tauber *et al.*, 2007). These evidences supports the strong genetic component in the diapause-induction mechanism and confirms a pre-determined dimension of diapause's induction. In biochemistry, some promising sensitive high-throughput methods permitting to measure major energetic components already exist (Foray *et al.*, 2012) and could be used for measuring metabolites associated with diapause phenotype. Gaining insights into genetic and biochemical processes could provide us a deeper understanding of diapause-induction mechanism.

4.6 Conclusion

In summary, while observing intra-genus variation of diapause-induction mechanisms, the environmental cues used by the studied species to induce diapause were mirrored by their thermal niche (Fig. 10). This supports the importance of a specific diapause-induction mechanism in order to survive specific environmental conditions (Schmidt & Paaby, 2008) and underpins the vital importance of a finely tuned diapause-induction mechanism (Kyriacou *et al.*, 2008). Furthermore, I could detect a phylogenetic signal between sister species *S. cynipsea* and *S. neocynipsea,* with both species being associated with high-altitude environments, remembering us that the diapause-induction mechanisms are conserved for some time even if the process is under strong selection. In the context of changing climatic conditions, there is a need to gain more insights about induction threshold of diapause-induction mechanisms. I counsel further studies to follow a similar experimental setup with an increased number of treatments (Xue *et al.*, 2002). These experiments were conducted in the lab and could be inconsistent with selection process occurring in the field (Harshman & Hoffmann, 2000), further field studies conducted under semi-natural conditions would allow to better understand species' phenology (Hoffmann, Scott, Partridge, & Hallas, 2003).

Acknowledgements

What an amazing year!

I would like to express my gratitude to my supervisor Wolf U. Blanckenhorn for his friendly guidance and support through the learning process of this master thesis. Special thanks to Patrick Rohner for his impressive availability and help. Furthermore, I would like to thanks Martin A. Schäfer and Kevin Vega for many good advices and helpful reviewing and the rest of the Blanckenhorn research group for their kindness and inspiring discussions. Last but not least, my thoughts go to my family and friends for their great support.

Thank you!

References

Altermatt F. **2010**. Climatic warming increases voltinism in European butterflies and moths. *Proceedings of the Royal Society B: Biological Sciences* **277**: 1281–1287.

Ang Y, Meier R. **2010**. Five additions to the list of sepsidae (Diptera) for vietnam: Perochaeta cuirassa sp. n., Perochaeta lobo sp. n., Sepsis spura sp. n., Sepsis sepsi Ozerov, 2003 and Sepsis monostigma Thompson, 1869. *ZooKeys* **70**: 41–56.

Bateman A. **1948**. Intra-sexual selection in Drosophila. *Heredity* **2**: 349–368.

Baylac M, Villemant C, Simbolotti G. **2003**. Combining geometric morphometrics with pattern recognition for the investigation of species complexes. *Biological Journal of the Linnean Society* **80**: 89–98.

Bean, D. W., Beck SD. **1980**. The role of juvenile hormone in the larval diapause of the European corn borer, Ostrinia nubilalis. *Journal of Insect Physiology* **26**: 579–584.

Bell WJ, Bohm MK. **1975**. Oosorption in insects. *Biological Reviews* **50**: 373–396.

Bell RA, Rasul CG, Joachim FG. **1975**. Photoperiodic induction of the pupal diapause in the tobacco hornworm, Manduca sexta. *Journal of Insect Physiology* **21**: 1471–1480.

Bewick V, Cheek L, Ball J. **2005**. Statistics review 14: Logistic regression. *Critical care (London, England)* **9**: 112–118.

Bey I, Croci-Maspoli M, Fuhrer J, Kull C, Appenzeller C, Knutti R, Schär CJ. **2011**. Swiss Climate Change Scenarios CH2011.

Blanckenhorn W. **1998**. Altitudinal differentiation in the diapause response of two species of dung flies. *Ecological Entomology*: 1–8.

Bradshaw WE, Holzapfel CM. **2007**. Evolution of Animal Photoperiodism. *Annual Review of Ecology, Evolution, and Systematics* **38**: 1–25.

Chown SL, Hoffmann AA, Kristensen TN, Angilletta MJ, Stenseth N, Pertoldi C. **2010**. Adapting to climate change: A perspective from evolutionary physiology. *Climate Research* **43**: 3–15.

Danks H V. **1987**. *Insect Dormancy: An Ecological Perspective*.

Danks H V. 1994. Diversity and integration of life-cycle contol in insects. *Insect life-cycle polymorphism.*: 97–104.

Danks H V. **2002**. The range of insect dormancy responses. *European Journal of Entomology*

99: 142.

Demont M, Blanckenhorn WU. **2008**. Genetic differentiation in diapause response along a latitudinal cline in European yellow dung fly populations. *Ecological Entomology* **33**: 197–201.

Denlinger D. **1972**. Induction and termination of pupal diapause in Sarcophaga (Diptera: Sarcophagidae). *The Biological Bulletin* **142**: 11–24.

Denlinger L. **1979**. Pupal diapause in tropical flesh flies: environmental and endocirne regulation, metabolic rate and genetic selection. *Bio. BUll*: 31–46.

DeWitt TJ, Sih A, Wilson DS. **1998**. Costs and limits of phenotypic plasticity. *Trends in Ecology and Evolution* **13**: 77–81.

Foray V, Pelisson PF, Bel-Venner MC, Desouhant E, Venner S, Menu F, Giron D, Rey B. **2012**. A handbook for uncovering the complete energetic budget in insects: The van Handel's method (1985) revisited. *Physiological Entomology* **37**: 295–302.

Garland T. **2014**. Trade-offs. *Current Biology* **24**: R60–R61.

Gomi T. **1997**. Geographic variation in critical photoperiod for diapause induction and its temperature dependence in Hyphantria cunea Drury (Lepidoptera: Arctiidae). *Oecologia* **111**: 160–165.

Hairston NG, Kearns CM. **1995**. The interaction of photoperiod and temperature in diapause timing: A copepod example. *Biological Bulletin* **189**: 42–48.

Harshman L, Hoffmann A. **2000**. Laboratory selection experiments using Drosophila: what do they really tell us? *Trends in ecology & evolution (Personal edition)* **15**: 32–36.

Hastings MH. **2001**. Adaptation to seasonal change: photoperiodism and its mechanism. *Journal of biological rhythms* **16**: 283–301.

Hoffmann AA, Scott M, Partridge L, Hallas R. **2003**. Overwintering in Drosophila melanogaster: outdoor field cage experiments on clinal and laboratory selected populations help to elucidate traits under selection. *Journal of Evolutionary Biology* **16**: 614–623.

Hopper KR. **1999**. Risk-Spreading and Bet-Hedging in Insect Population Biology 1. *Annual Review Entomology* **44**: 535–560.

Hosmer DW, Lemeshow S. **2000**. *Applied Logistic Regression. 2nd ed.*

Huey RB, Kingsolver JG. **1989**. Evolution of thermal sensitivity of ectotherm performance. *Trends in ecology & evolution (Personal edition)* **4**: 131–135.

Janzen FJ, Stern HS. **1998**. Logistic regression for empirical studies of multivariate selection. *Evolution* **52**: 1564–1571.

Kivelä SM, Välimäki P, Gotthard K. **2013**. Seasonality Maintains Alternative Life-History Phenotypes. *Evolution* **67**: 3145–3160.

Koštál V. **2006**. Eco-physiological phases of insect diapause. *Journal of Insect Physiology* **52**: 113–127.

Kraushaar U, Goudet J, Blanckenhorn WU. **2002**. Geographical and altitudinal population genetic structure of two dung fly species with contrasting mobility and temperature preference. *Heredity* **89**: 99–106.

Kyriacou CP, Peixoto AA, Sandrelli F, Costa R, Tauber E. **2008**. Clines in clock genes: fine-tuning circadian rhythms to the environment. *Trends in genetics : TIG* **24**: 124–32.

Mousseau, T. A., & Dingle H. **1991**. Maternal effects in insect life histories. *Annual review of entomology* **36**: 511–534.

Ozerov AL. **2005**. *World catalogue of the family Sepsidae (Insecta: Diptera)*.

Parker GA. **1972**. Reproductive Behaviour of *Sepsis Cynipsea* (L.) (Diptera: Sepsidae) the Significance of the Precopulatory Passive Phase and Emigration. *Behaviour* **41**: 242–250.

Peters R. **1985**. *The ecological implications of body size* (CU Press., Ed.). Cambridge University Press.

Pont AC, Meier R. **2002**. *The Sepsidae (Diptera) of Europe*.

Puniamoorthy N. Morphology, molecules and mating behavior: an integrative study of population diverfence and speciation in widepread sepsid flies (Sepsidae, Diptera)., PhD Dissertation.

Puniamoorthy N, Ismail MRB, Tan DSH, Meier R. **2009**. From kissing to belly stridulation: comparative analysis reveals surprising diversity, rapid evolution, and much homoplasy in the mating behaviour of 27 species of sepsid flies (Diptera: Sepsidae). *Journal of Evolutionary Biology* **22**: 2146–2156.

Roff DA. **1983**. Phenological adaptation in a seasonal environment: a theoretical perspective. *Diapause and life cycle strategies in insects*: 253–270.

Rohner PT, Bächli G, Pollini Paltrinieri L, Duelli P, Obrist MK, Jochmann R, Blanckenhorn WU. **2015**. Distribution, diversity gradients and Rapoport's elevational rule in the black scavenger flies of the Swiss Alps (Diptera: Sepsidae). *Insect Conservation and Diversity* **8**: 367–376.

Saunders DS. **1971**. The temperature-compensated photoperiodic clock 'programming'development and pupal diapause in the flesh-fly, Sarcophaga argyrostoma.

Journal of Insect Physiology **17**: 801–812.

Scharf I, Bauerfeind SS, Blanckenhorn WU, Schäfer M a. 2010. Effects of maternal and offspring environmental conditions on growth, development and diapause in latitudinal yellow dung fly populations. *Climate Research* **43**: 115–125.

Schmidt PS, Matzkin L, Ippolito M, Eanes WF. **2005a**. Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. *Evolution; international journal of organic evolution* **59**: 1721–1732.

Schmidt PS, Paaby AB. **2008**. Reproductive Diapause and Life-History Clines in North American Populations of *Drosophila Melanogaster*. *Evolution* **62**: 1204–1215.

Schmidt PS, Paaby AB, Heschel MS. **2005b**. Genetic variance for diapause expression and associated life histories in *Drosophila melanogaster*. *Evolution; international journal of organic evolution* **59**: 2616–2625.

Schmidt PS, Zhu C-T, Das J, Batavia M, Yang L, Eanes WF. **2008**. An amino acid polymorphism in the couch potato gene forms the basis for climatic adaptation in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 16207–16211.

Schmidt, P. S., & Conde DR. **2006**. Environmental Heterogeneity and the Maintenance of Genetic. *Evolution* **60**: 1602–1611.

Snyder BF, Gowaty PA. **2007**. A Reappraisal of Bateman's Classic Study of Intrasexual Selection. *Evolution* **61**: 2457–2468.

Stephens GC. **1968**. Insect photoperiodism. *Journal of Invertebrate Pathology* **11**: 152–153.

Tang-Martinez Z, Ryder TB. **2005**. The Problem with Paradigms: Bateman's Worldview as a Case Study. *Integrative and comparative biology* **45**: 821–830.

Tatar M, Yin CM. **2001**. Slow aging during insect reproductive diapause: Why butterflies, grasshoppers and flies are like worms. *Experimental Gerontology* **36**: 723–738.

Tauber MJ, Tauber CA, Masaki S. **1986**. *Seasonal adaptations of insects*.

Tauber E, Zordan M, Sandrelli F, Pegoraro M, Osterwalder N, Breda C, Daga A, Selmin A, Monger K, Benna C, *et al.* 2007. Natural Selection Favors a Newly Derived timeless Allele in *Drosophila melanogaster*. *Science* **316**: 1895–1898.

Taylor F. **1980**. Optimal switching to diapause in relation to the onset of winter. *Theoretical population biology* **18**: 125–133.

Teuschl Y, Blanckenhorn WU. **2007**. The reluctant fly: what makes *Sepsis cynipsea* females

willing to copulate? *Animal Behaviour* **73**: 85–97.

Teuschl Y, Reim C, Meister B, Egger J, Blanckenhorn WU. **2010**. Strategic Ejaculation in the Black Scavenger Fly *Sepsis cynipsea* Revisited: Copula Duration as a Function of Sperm Depletion and Body Size. *Ethology* **116**: 1118–1126.

Tobin PC, Nagarkatti S, Loeb G, Saunders MC. **2008**. Historical and projected interactions between climate change and insect voltinism in a multivoltine species. *Global Change Biology* **14**: 951–957.

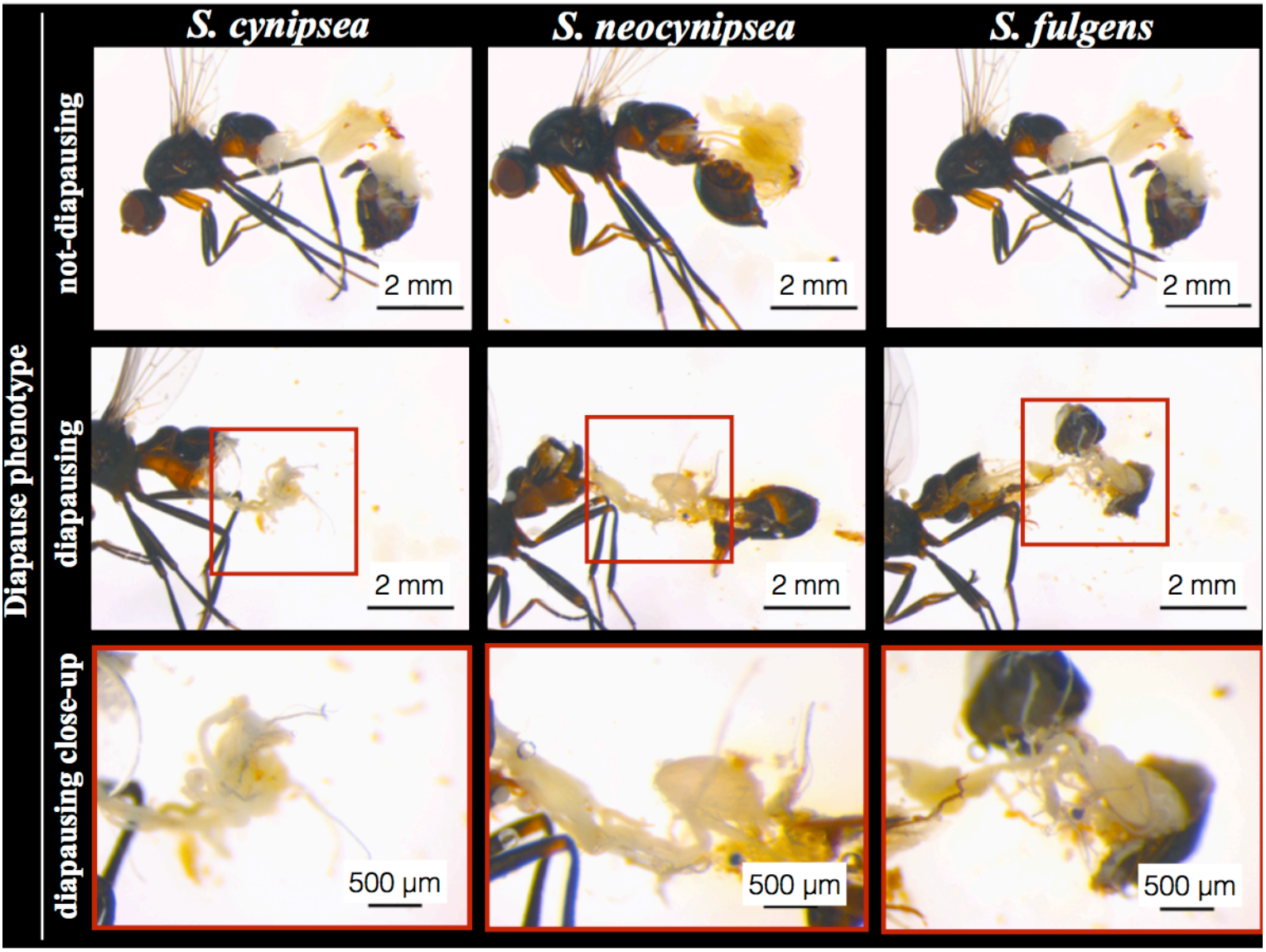
Wilde J. **1962**. Photoperiodism in Insects and Mites. *Annual Review of Entomology* **7**: 1–26.

Williams KD, Sokolowski MB. **1993**. Diapause in *Drosophila melanogaster* females: a genetic analysis. *Heredity* **71**: 312–317.

Xue F, Spieth HR, Aiqing L, Ai H. **2002**. The role of photoperiod and temperature in determination of summer and winter diapause in the cabbage beetle, *Colaphellus bowringi* (Coleoptera: Chrysomelidae). *Journal of insect physiology* **48**: 279–286.

Zhao L, Annie ASH, Amrita S, Yi SKF, Rudolf M. **2013**. Does better taxon sampling help? A new phylogenetic hypothesis for Sepsidae (Diptera: Cyclorrhapha) based on 50 new taxa and the same old mitochondrial and nuclear markers. *Molecular phylogenetics and evolution* **69**: 153–64.

Appendix


S. cynipsea
S. neocynipsea
S. fulgens
Diapause phenotype
not-diapausing
diapausing
diapausing close-up
2 mm
500 µm

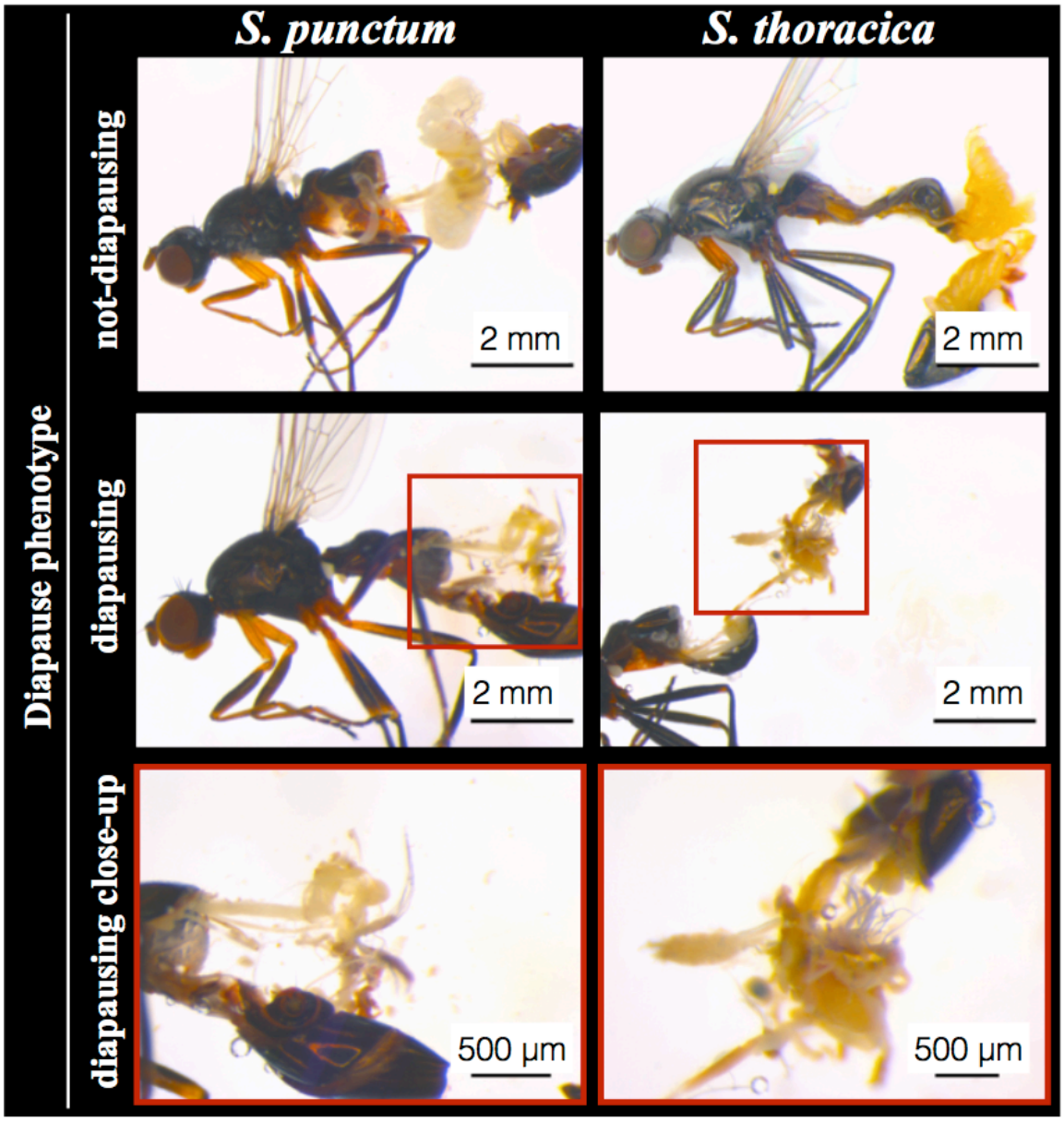


Figure A1: Diapause phenotypes for the five different species studied. Eggs are the white/yellow bodies and clearly visible in all the "not-diapausing" phenotypes. Inversely, in the "diapausing" phenotypes, no eggs are visible. Instead we can distinguish in the "diapausing close-up" gray threads which are the atrophied ovarioles.

Table A1: Multiple logistic regression of diapause incidence among studied Sepsid species in relation to temperature and photoperiod and the resulting interaction. Reference levels for temperature and photoperiod and species were respectively: warm (18°C), short (8 h light:16 h dark) and *S. cynipsea*. Key for species: n: *S. neocynipsea*; f: *S. fulgens*; p: *S. punctum*; t: *S. thoracica*. Significant p-values are in bold, N: total sample size per species.

source	df	χ2	P
photo(1)	1	0.017	0.895
sp	4	85	**<0.001**
sp(n)	1	33.375	**<0.001**
sp(f)	1	0.017	0.895
sp(p)	1	40.649	**<0.001**
sp(t)	1	3.5	0.061
temp(1)	1	21.527	**<0.001**
photo(1) x temp (1)	1	0.681	0.409
photo x sp	1	24.207	**<0.001**
photo(1) x sp (n)	1	10.863	**<0.001**
photo(1) x sp (f)	1	0.056	0.813
photo(1) x sp (p)	1	8.812	**0.003**
photo(1) x sp (t)	1	0.577	0.447
sp x temp	4	8.969	0.062
sp (n) temp (1)	1	4.416	**0.036**
sp (f) temp (1)	1	0.541	0.462
sp (3) temp (1)	1	0.783	0.376
sp (4) temp (1)	1	0.651	0.42
photo x sp x temp	4	25.379	**<0.001**
photo (1) x sp (n) x temp (1)	1	5.928	**0.015**
photo (1) x sp (f) x temp (1)	1	3.808	**0.051**
photo (1) x sp (p) x temp (1)	1	4.139	**0.042**
photo (1) x sp (t) x temp (1)	1	0.279	0.597
N	919		

Table A2: Outbreeding manipulations were conducted before the experiments. All populations originate from Switzerland.

species	origin of the population	number of lines crossed	altitude (m)	latitude (°N)	longitude (°E)
Sepsis cynipsea	Wolzeralp	2	759	47.23	9.17
Sepsis cynipsea	Zürich	2	408	47.34	8.54
Sepsis cynipsea	Sörenberg	2	884	46.87	8.27
Sepsis neocynipsea	Oberwald	1	1377	46.58	8.44
Sepsis neocynipsea	Geschinen	1	1370	46.49	8.27
Sepsis neocynipsea	Hospental	1	1493	46.62	8.57
Sepsis neocynipsea	Zürich	1	408	47.34	8.54
Sepsis neocynipsea	Wolzenalp	1	759	47.23	9.17
Sepsis neocynipsea	Sörenberg	1	884	46.87	8.27
Sepsis fulgens	Zürich	2	408	47.34	8.54
Sepsis fulgens	Bignasco	1	443	46.35	8.62
Sepsis fulgens	Piotta	1	990	46.52	8.68
Sepsis fulgens	Maggia	1	372	46.25	8.7
Sepsis fulgens	Moschberg	1	745	46.9	7.63
Sepsis punctum	Zürich	2	408	47.34	8.54
Sepsis punctum	Lenzerheide	2	1473	46.75	9.55
Sepsis thoracica	Santa-Maria	2	1375	46.11	8.92
Sepsis thoracica	Zürich	4	408	47.34	8.54

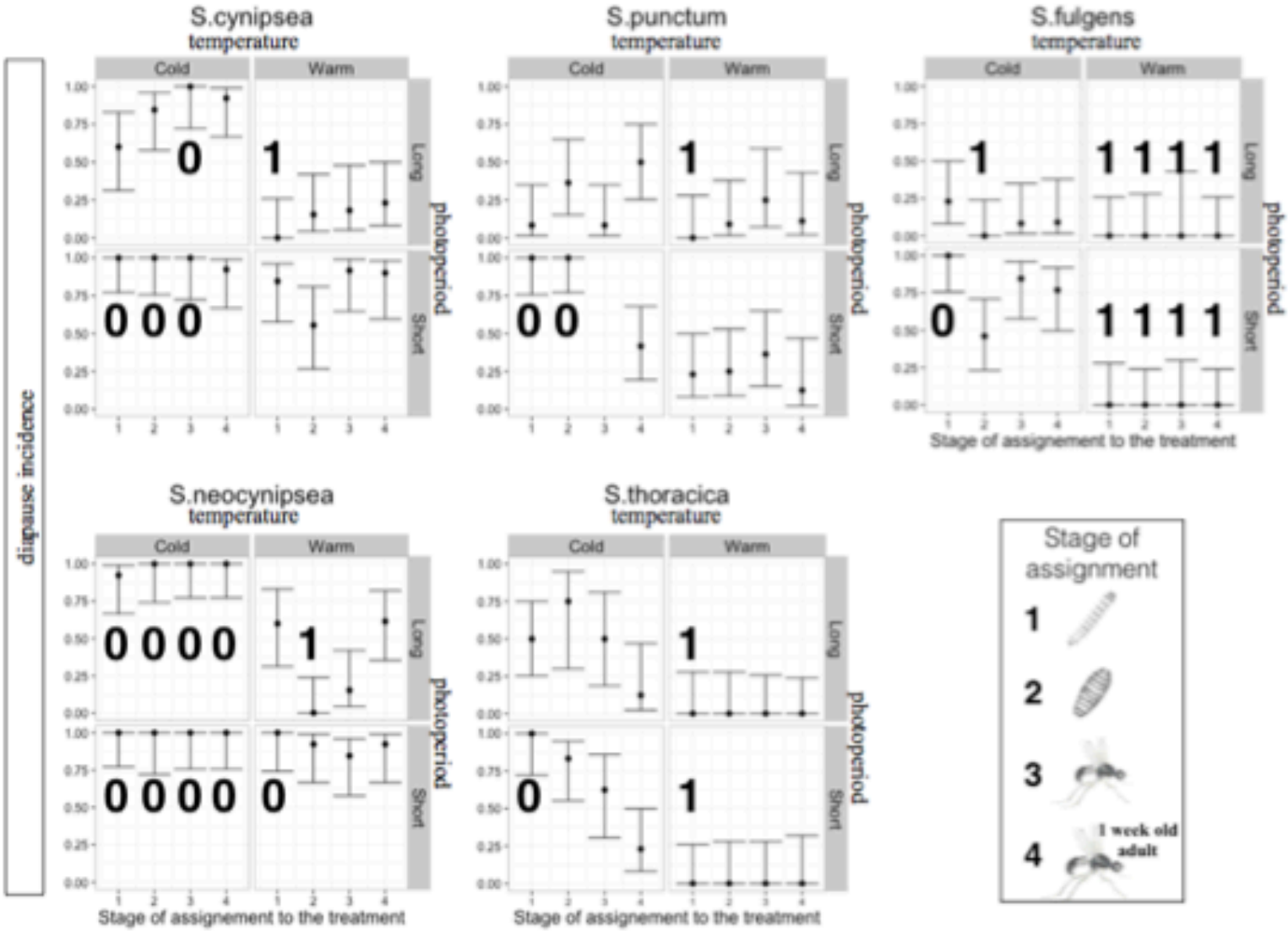


Figure A2: Variance was added to subsets with no variation. Figure above data-point indicates "0" or "1" (single values) that were added before conducting the statistical analysis. In total, 31 data-points were added.

Appendix II

Complementary information for the interpretation of Table 3

χ2 are the Wald chi-squared values, P is the 2-tailed p-value as df columns give the degrees of freedom for each coefficient. Under the Source column, the row *stage* represent the test of the overall variable, which contains four levels – the four life stages assignments. A significant value of the life stage main effect means one or many life stages were differing from one another. But, this do not specify, which life stage differs from the others. This information can be obtained by looking at the graphical analysis (Fig.7). Always under the Source column, the lines stage (1), stage (2) and stage (3) use the larval stage as reference value and inform us if there is a significant difference between the pupal stage and the larval stage (in the table: stage (1)), the adult stage and the larval stage (in the table: stage (2)) and the one week old adult and the larval stage (in the table: stage (3)). E.g.: in *S. thoracica* in the cold treatments, all three post pupal life stage assignment were significantly differing from the diapause incidence at the larval stage.

Statement of authorship

I declare that I have used no other sources and aids other than those indicated. All passages quoted from publications or paraphrased from these sources are indicated as such, i.e. cited and/or attributed. This thesis was not submitted in any form for another degree or diploma at any university or other institution of tertiary education.

Zurich, 04.01.2016

Valérian Zeender